EFFECTS OF AMMONIUM PERCHLORATE ON THE THYROID HORMONE LEVELS OF THE SPRAGUE-DAWLEY RAT

THESIS

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13. ABSTRACT (Maximum 200 words)

The purpose of this research was to determine the threshold dose for ammonium perchlorate (AP) in the Sprague-Dawley rat. No dose response data exist for AP and the EPA has studied literature on the subject of perchlorates to determine a provisional reference dose. The Perchlorate Group, a consortium of DoD and industry representatives, believes this provisional reference dose is too conservative. This experiment was executed to provide dose response data on which to base a more accurate reference dose. The study consisted of eight groups of 12 Sprague-Dawley rats, six male and six female, which were exposed to incremental doses of AP in their drinking water.
The results indicated that Triiodothyronine (T3) levels in male and female rats fell. Thyroxine (T4) levels in male rats remained relatively unchanged. Reverse Triiodothyronine (rT3), Thyrotropin (TSH), and Thyroglobulin (Tg) all increased in both males and females. These results imply that the AP anion blocked the uptake of iodine in the thyroid. Although an estimated threshold could not be determined, the NOAEL for AP on the TSH levels was .44 mg/kg/day for the male rats and .124 mg/kg/day for the female rats. These NOAELs are consistent with the assumptions made by the EPA, which estimated a NOAEL of .14 mg/kg/day for perchlorates.

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The views expressed in this thesis are those of the author and do not reflect the official policy or position of the Department of Defense or the U.S. Government.

Preface

The purpose of this study was to determine the estimated threshold dose of ammonium perchlorate (AP) in the Sprague Dawley rat. This threshold dose could then aid the EPA, Department of Defense, NASA, and their contractors in determining a human threshold dose and subsequently, clean-up levels of AP released into the environment. The threshold could not be determined because the data did not allow for an adequate fit to the sigmoidal function. However, a NOAEL for AP's affect on TSH levels was determined to be .44 mg/kg/day in male rats and .124 mg/kg/day for female rats. The NOAELs were determined by exposing rats to incremental doses of AP in their drinking water for two weeks. At the completion of the exposure period, the rats were sacrificed, blood drawn and their thyroid hormone levels measured using radioimmunoassay. Univariate, multivariate analysis, and maximum likelihood estimation were then implemented in an attempt to determine the threshold dose for the exposed rats.

This research effort would not have been possible without the contribution and support of many individuals. I would like to express special thanks to my faculty advisor, Dr. Daniel J. Caldwell whose knowledge and experience made this and outstanding learning experience. I would also like to thank Ed Kinkead and Robin Wolfe for their instruction in the "Pathtox" software, Brenda Schimmel for her instruction in animal handling, Peggy Parrish and Jerry Nichols for their expertise and instruction in necropsy, Latha Narayanan for her expertise with the radioimmunoassays, Lt Col Michael Shelley for his instruction in risk analysis, and Dan Reynolds and Carlyle Flemming for their instruction in the statistical realm. I would also like to give special thanks to my wife, Cheryl, and daughters, Jennifer, Kathleen and Rachel, for their love and support throughout the thesis effort and master degree program.

James H. King, Jr.

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EFFECTS OF AMMONIUM PERCHLORATE ON THE THYROID HORMONE LEVELS OF THE SPRAGUE-DAWLEY RAT

I. Introduction

Objective

The primary objective of this research is to determine toxicity information to establish permissible exposure levels of ammonium perchlorate.

Background

Ammonium perchlorate (AP), [NH4+] [ClO4-], is a white, crystalline solid anion which is used as an oxidant in solid propellants for rockets and missiles. It is also used in explosive mixtures, mines, shells, timing devices, other pyrotechnics, and as a chemical raw material (CPIA, 1989). In addition to being listed by the Environmental Protection Agency (EPA) as a class B2 carcinogen (Table 1-1), when AP is used as an oxidizer in solid propellants, 20% of the exhaust produced is HCl by weight. Therefore, clean propellants are being sought to replace AP because of the impact of large quantities

Group		Criteria for Classification
A	-Human carcinogen	-Sufficient evidence from epidemiologic studies
B	-Probable human carcinogen (two subgroups)	-Limited evidence from epidemiologic studies and sufficient evidence from animal studies (B1); or inadequate evidence from epidemiologic studies (or no data) and sufficient evidence from animal studies (B2)
C	-Possible human carcinogen	-Limited evidence from animal studies and no human data
D	-Not classifiable as to human carcinogenicity	-Inadequate human and animal data or no data
E	-Evidence of noncarcinogenicity in humans	-No evidence of carcinogenicity from adequate human and animal studies

Table 1-1. Categorization of Evidence of Carcinogenicity
SOURCE: Science and Judgment in Risk Assessment (1994) adapted from EPA, 1987a

Traditionally, a RfD is associated with non-carcinogens. With the exception of carcinogens, it is generally assumed that every organism has the capacity to adapt to or otherwise tolerate some exposure to any substance until a threshold amount is reached. With regard to carcinogens, modern dose-response models are based on the premise that exposure to even one molecule of a carcinogen poses a small but non-zero increased risk of tumor formation (S&J, 1994). What sets AP (and other agents which inhibit iodide uptake by the thyroid gland) apart from other B2 carcinogens is the mechanism of action which leads to the toxic response of an excessive development of the thyroid (hypertrophy), an abnormal increase in the number of tissue cells (hyperplasia), followed by the formation of tumors (neoplasia). It is not exposure to the chemical itself which leads to hyperplasia, but the body's physiological response to the chemical. AP blocks iodide uptake, which leads to a decrease in blood hormone levels of Thyroxine (T4) and Triiodothyronine (T3). Subsequently, the pituitary gland increases production of thyroid stimulating hormone (TSH). This chain of events has been shown to elicit the toxic response of hypertrophy, followed by hyperplasia, which is then followed by neoplasia (Hill et al., 1989; Paynter et al., 1988). Therefore, if a threshold can be found for which there is no decrease in T3 or T4 levels followed by an increase in TSH levels, carcinogenesis should not occur.

Dollarhide (1992) based her provisional RfD on a study conducted in 1952 using patients suffering from Graves' disease, a disease characterized by the enlargement of the thyroid gland and its increased metabolic rate (Stanbury and Wyngaarden). These patients were administered potassium perchlorate (KP) under various conditions. The

alone, the U.S. Air Force spent $80 million using a pump-and-treat method to return TCE to acceptable levels (Hurley, 1995).

In order for the EPA to establish a more accurate RfD for AP, dose-response data is needed (Dollarhide, 1992). Since there is minimal dose response data for AP in the literature, this requirement provides the basis for this research.

This thesis will analyze data from a 14-day acute study using Sprague-Dawley rats to acquire dose-response data on AP. This data will be analyzed using univariate and multivariate analysis and maximum likelihood estimation techniques to estimate the threshold dose for AP in the rat.

Possible Benefits

The dose-response data derived from this research could be used by the EPA to more accurately characterize a RfD dose for AP. Since it is now known that the rat thyroid is more sensitive than the human (Capen, 1992), this threshold would not need to be adjusted with the same conservatism as the provisional RfD (i.e. there would be no need to divide the threshold by 1000 to derive the RfD). If AP concentrations in groundwater or contaminated soil did not have to be reduced to such low levels, which are overly conservative because of insufficient information, the DoD, NASA and their contractors could save a significant amount of resources which could be utilized elsewhere.

Overview

This thesis consists of four more chapters. The following chapter is a review of the literature concerning AP and the mechanism by which it exerts its effect. Chapter

II. Literature Review

The purpose of the literature review is to emphasize the need for this research by describing the lack of dose response data for AP. Furthermore, it is intended to familiarize the reader with the mechanism by which thyroid hormone levels are inhibited. Hormones are products of living cells that circulate in body fluids and produce a specific effect on the activity of cells remote from their point of origin. This inability of the thyroid gland to produce thyroid hormones, if not corrected, leads to excessive development of thyroid tissue cells (hypertrophy), an unusual increase in the number of tissue cells (hyperplasia) and the formation of abnormal masses of tissue (tumors) that possess no physiologic function (neoplasia) in experimental rodents (Capen, 1992; Hill et al., 1989; Paynter et al., 1988).

The Thyroid Gland

This section explains the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated.

Thyroxine (T4) and triiodothyronine (T3) are classically regarded as the two hormones produced by the thyroid gland. They contain 4 and 3 atoms of iodine, respectively, and are abbreviated as T4 and T3 because of their iodine content (Fig. 2-1). The thyroid hormones are synthesized in the thyroid gland by iodinating thyroglobulin (Tg), an iodine containing protein stored in the thyroid (Goodman and van Middlesworth, 1980). The first stage in the synthesis of thyroid hormones is the uptake of iodide from the blood by the thyroid gland (Fig. 2-2).

thyroid is active (250:1). Iodide uptake may be blocked by several anions, one of which is perchlorate (Goodman and van Middlesworth, 1980).

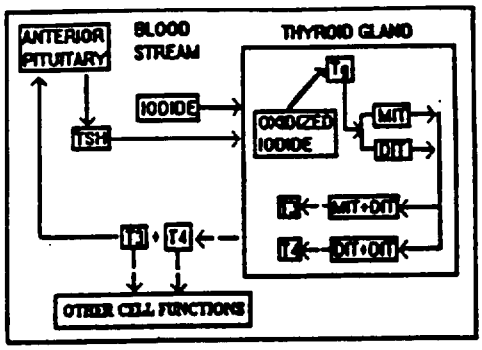

Figure 2-2. Schematic representation of thyroid hormone biosynthesis and secretion. Source: Adapted from Stevens (1985).

T4 is the major hormone secreted from the thyroid and is converted to more active T3 in a variety of peripheral tissues, including the pituitary gland. T4 is also metabolized to rT3 (Fig. 2-1) which is hormonally inactive and has no know function, except perhaps as an inhibitor of the conversion of T4 to T3 (Hill et al., 1989; Stevens, 1985, Goodman and van Middlesworth, 1980).

Homeostatic control of thyroid hormone synthesis and secretion in the thyroid gland is effected by a sensitive feedback mechanism that responds to changes in circulating levels of the thyroid hormones T4 and T3. The mechanism involves the anterior pituitary of the brain (Fig. 2-3) (Hill et al., 1989; Paynter et al., 1988; Houk, 1980). Thyroid-stimulating hormone (TSH, thyrotropin), which is secreted by the anterior pituitary gland and causes the thyroid to create new thyroid hormones, is very important in the feedback mechanism. It independently promotes iodine trapping and iodination of Tg. The rate of release of TSH from the pituitary is controlled by the circulating levels of T4 and T3.

mental retardation, suggesting that the hormones must be present during critical periods in order for normal development to occur.

Thyroid Gland Neoplasia

Hill et al. explains that thyroid neoplasia may be induced by exposure of experimental animals to a variety of treatment regiments, chemicals produced outside the body (exogenous), or physical agents. "It has been recognized for some time that neoplasms induced in experimental animals by a number of these treatments result from thyroid gland dysfunction, in particular, [enlargement of the thyroid gland and increased metabolic rate] hypothyroidism." Factors inducing hypothyroidism include iodine deficiency, surgically removing part of the thyroid gland, and the transplantation of TSH-secreting pituitary tumors. "The one factor common to each of these conditions is that they all lead to increased production of TSH and prolonged stimulation of the thyroid gland by "excess" TSH." Whatever the cause (i.e. low iodine diet, blocked iodide uptake by an anion), prolonged stimulation of the thyroid-pituitary feedback mechanism that results in the release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia. However, thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone or by surgically removing the pituitary gland (hypophysectomy) (Hill et al., 1989).

A recent review of chemical injury of the thyroid (Capen, 1992) showed that rodents treated with agents that directly interfere with thyroid hormone production in the thyroid gland depress T3 and T4 levels resulting in a compensatory increase of TSH.

level doses (Dollarhide, 1992). No dose-response data exist.

4) The rat is now considered to be more sensitive to thyroid hormone fluctuations than is man (Capen, 1992, Hill et al., 1989). The provisional RfD assumed the opposite.
5) The application of standard default uncertainty factors (i.e., division of the apparent safe level by 1000) by the EPA appears unjustified (Dollarhide, 1992). The biochemical mechanism by which perchlorate exerts its effect in humans is well understood, alleviating the need for application of safety factors normally associated with unknown xenobiotics. (Caldwell, 1995)

Human Data. Brabant et al. conducted a study in which 5 healthy males were exposed to an oral treatment of 300 mg of perchlorate 3 times daily over a 4-week period. Mean serum TSH levels actually decreased slightly and the thyroid volumes were unaltered. The body weights of the volunteers were not provided. However, using the standard 70 kg default body weight for risk assessment leads to a dose of 12.86 mg/kg/day. This would suggest that the threshold in healthy humans is higher than 12.86 mg/kg/day.

Burgi et al. (1974) administered 200 mg of perchlorate 3 times daily to three healthy females and two healthy males for 8 days. The average dose for the females was 11.04 mg/kg/day and the average for the males was 8.22 mg/kg/day. These doses were sufficient to completely block iodide uptake by the thyroid as measured in the urine. However, thyroid hormone levels were not measured in order to determine if this dose produced a decrease in T3 and T4 or and increase in TSH levels.

These two studies contradict the mechanistic conceptual model. Brabant et al. found that a dose of 12.86 mg/kg/day did not increase TSH levels while Burgi et al. found that a lower dose of 8.22 mg/kg/day was sufficient to completely block iodide uptake by the thyroid.

Exposure Conditions	Conclusions
4200 mg/kg (once)	-LD_{50}
650 mg/kg/day for one month	-No noticeable cumulative properties
190 mg/kg/day for three months	-Affects the regulation of the involuntary nervous system -Causes a statistically reliable change in the protein fractions of the blood serum -Disrupts the liver's ability to produce glycogen for carbohydrate storage

Table 2-2. Results of Experiments on 'white rats'
(Adapted from Shigan, 1963)

These results do not provide any insight in determining a threshold because there were no doses given at low concentrations.

Mannisto et al. (1979) studied the effects of Potassium Perchlorate (KP) on the thyroid of the Sprague-Dawley rat. He found that doses of KP from 7.6 to 15.3 mg/kg/day administered over a 4 day period reduced serum triiodothyronine (T3) and thyroxin (T4) levels and increased thyroid stimulating hormone (TSH) levels.

Conclusion

These experiments do not provide enough information on which to base an accurate RfD. Therefore, in an effort to provide more toxicological evidence, Caldwell (1995) has designed a study in which thyroid hormone levels and thyroglobulin (Tg) levels in the Sprague-Dawley rat were determined. This study provided specific dose-response data, from which a threshold level for thyroid hormone effects of AP can be determined. Since the EPA based their provisional perchlorate RfD on the Stanbury and Wyngaarden study and predicted that chronic administration of perchlorate at this dose would likely have resulted in lowering of the patients T3 and T4 levels, with subsequent

III. Methodology

Introduction

This section describes the process by which this research was conducted. The design will be explained, followed by a description of the execution and the analysis techniques. It concludes with a summary of the chapter.

Design

The 14-day pilot study included 96 rats, 48 male and 48 female. They were divided into eight dose groups, including a control (Table 3-1). The male rats were estimated to consume water at the rate of 45 ml/day/rat and have an estimated body weight of 450 grams. The female rats were estimated to consume water at the rate of 27 ml/day/rat and have an estimated body weight of 270 grams. The target doses are specified in Table 2. These target doses were designed around an estimated threshold dose of 10 mg/kg/day based on the available literature (Caldwell, 95).

Group	No. of Animals		AP Conc. (mg/L)	AP Target Dose (mg/kg/day)
	Males	Females		
Control	6	6	0.00	0.0
Very Low	6	6	1.25	0.125
Low	6	6	5.00	0.5
Med. Low	6	6	12.50	1.25
Medium	6	6	25.00	2.5
Med. High	6	6	50.00	5.0
High	6	6	125.00	12.5
Very High	6	6	250.00	25.0

Table 3-1. Pilot Study Dose Groups, Concentrations and Target Doses
Source: Caldwell (1995).

Statistical Analysis

The results were analyzed using two-factor Analysis of Variance (ANOVA) (Statistix 4.1), Multivariate Analysis of Variance (MANOVA) (SAS), Tukey's method for multiple comparisons (Statistix 4.1), and Maximum Likelihood Estimation (MLE) (SAS). Two-factor ANOVA was used to determine if there were statistically significant differences between dose groups and the male and female rats. Two-factor MANOVA was used to determine if there were statistically significant differences between dose groups and the male and female rats. If the males and females responded similarly to the dosing, Tukey's method for multiple comparisons was used to determine which dose groups differed.

Capen (1992) concluded that if a dose can be found for which there is no decrease in T3 or T4 accompanied by an increase in TSH, that dose can be considered the threshold. Therefore, the sigmoid function was used to fit the data points for T3 and TSH. The sigmoid function was not used to fit the T4 data because there was no dose response relationship. Maximum Likelihood Estimation (MLE) was then used to determine the parameter point estimates for the sigmoid function. Once the parameter estimates were determined, the function was evaluated using the F-test for lack of fit (LOF). If the functions passed the LOF test, this would mean that they accurately characterized the relationship between dose and hormone levels. These functions, which serve as conservative estimations of the dose-response relationship, could subsequently be used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

Problem 1 (Average Water Consumption)

(1) Does the dose of AP affect average water consumption?

(2) Does the sex of the rat affect average water consumption?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Problem 2 (Body Weight Gain)

(1) Does the dose of AP affect average body weight gain?

(2) Does the sex of the rat affect average body weight gain?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Problem 3 (Thyroid/Body Weight Ratio)

(1) Does the dose of AP affect thyroid/body weight ratio?

(2) Does the sex of the rat affect thyroid/body weight ratio?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Research Null Hypotheses:

Hypothesis 1 (Average Water Consumption)

(1) The dose of AP does not affect average water consumption.

(2) The sex of the rat does not affect average water consumption.

(3) There is no interaction between the dose of AP and the sex of the rat.

Hypothesis 2 (Body Weight Gain)

(1) The dose of AP does not affect body weight gain.

(2) The sex of the rat does not affect body weight gain.

(3) There is no interaction between the dose of AP and the sex of the rat.

(2) The sex of the rat has no affect Tg, T3, rT3, T4, and TSH thyroid hormone levels.

(3) There is no interaction between the dose of AP and the sex of the rat.

An answer which contradicted any of the three previous null hypotheses resulted in rejecting the respective null hypothesis.

Maximum Likelihood Estimation

Maximum likelihood estimation (MLE) was used to obtain point estimates of the parameters of the sigmoid function. The sigmoid function was used because it approaches a threshold affect at low doses (Fig. 3-1). The sigmoid function is as follows:

$$\text{HormLevel}(\text{Dose}, B0, B1) := \frac{B0 \cdot \text{Dose}^{B1}}{\left[\text{Dose}^{B1} + \left(\frac{B0}{2}\right)^{B1}\right]}$$

Where B0 is the highest observed response value (highest observed hormone level) and B1 is an exponent parameter which controls the function shape.

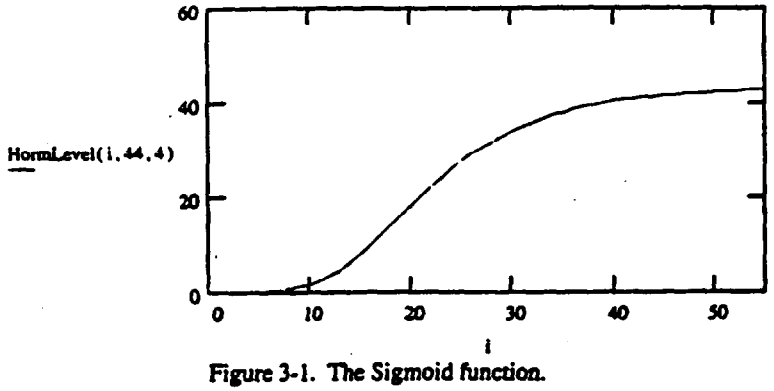

Figure 3-1. The Sigmoid function.

The MLE method assumes that all the hormone levels are normally distributed, have equal variances and are independent. The likelihood function:

$$F = \frac{MSLF}{MSPE}$$

The research problem for each data set was as follows:

Problem

Does the function accurately characterize the data?

Research Null Hypothesis

The function accurately characterizes the data.

If the value for the computed F is less than or equal to the critical value for the level of significance (α=.05), then the null hypothesis holds.

Tukey's Method for Multiple Comparisons

When the no-interaction hypothesis was not rejected and at least one of the two main effect null hypotheses was rejected, Tukey's method was used to identify significant differences between dose groups. For identifying differences among the means when the null hypothesis was rejected,

1. Obtain the value of the upper-tail α from the studentized t-distribution, above which the null is rejected (Q).
2. Compute $w = Q * (MSE/(JK))^{1/2}$, where MSE is the mean squared error obtained from the ANOVA table and JK is the number of observations averaged to obtain each of the sample means compared in step 3.
3. Order the sample means from smallest to largest and underscore all pairs that differ by less than w. Pairs not underscored correspond to significantly different levels for the factor under consideration.

IV. Data Description and Analysis

Introduction

This chapter presents and analyzes the raw data obtained from this study. Two-factor ANOVA was used to determine statistically significant differences between dose groups based on weight gain, water consumption, thyroid/body weight ratio. Two-factor MANOVA was used to determine significant differences between dose groups and sex based on thyroid hormone levels. When a null hypothesis was not rejected the analysis was terminated. However, when the no-interaction hypothesis was not rejected and at least one of the two main effect null hypotheses was rejected, Tukey's method was used to identify which levels differed from the control. Maximum Likelihood Estimation (MLE) was then used to determine the point estimates for the parameters of the sigmoid function used to fit the data. The F-test for lack-of-fit was then used to determine if the function accurately characterized the data.

The data was analyzed in this order. First, the water consumption data was analyzed to determine whether there was any statistically significant difference between dose groups and what the actual doses were. Next, body weight gain and thyroid/body weight ratio was analyzed to determine whether there was a difference between dose groups. The effect of dose, if any, on thyroid hormone levels was then evaluated. If an affect was noted, maximum likelihood estimation was used to maximize the parameters for the sigmoid function. The function was then used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

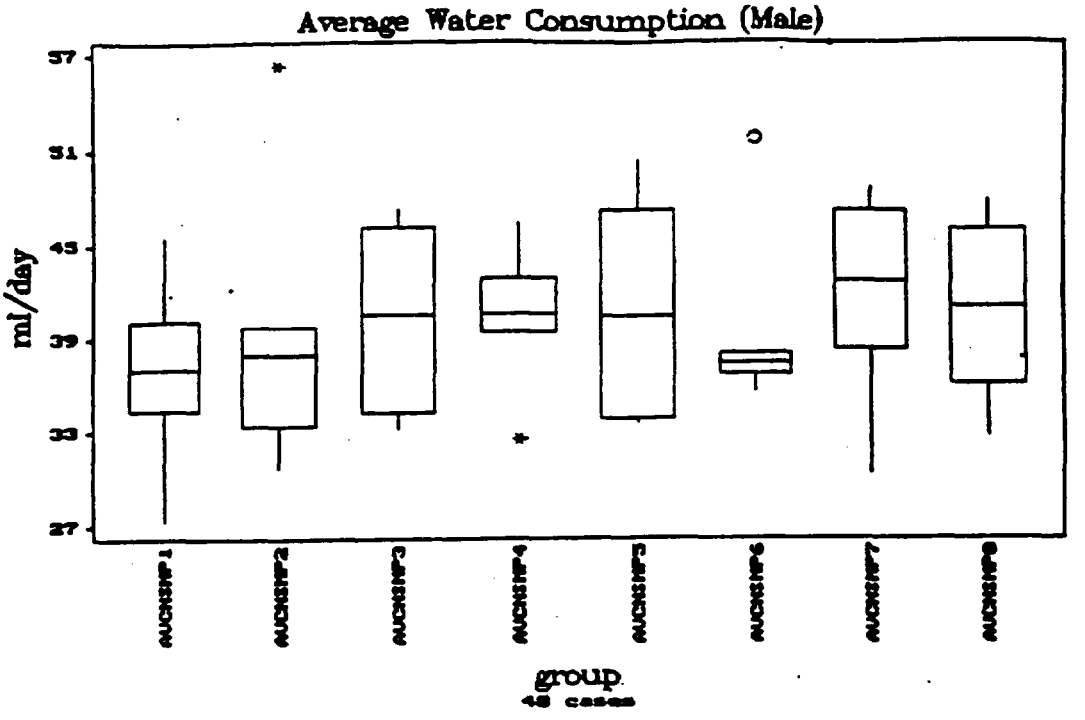

Figure 4-1. Boxplots of male rat average water consumption by dose group.

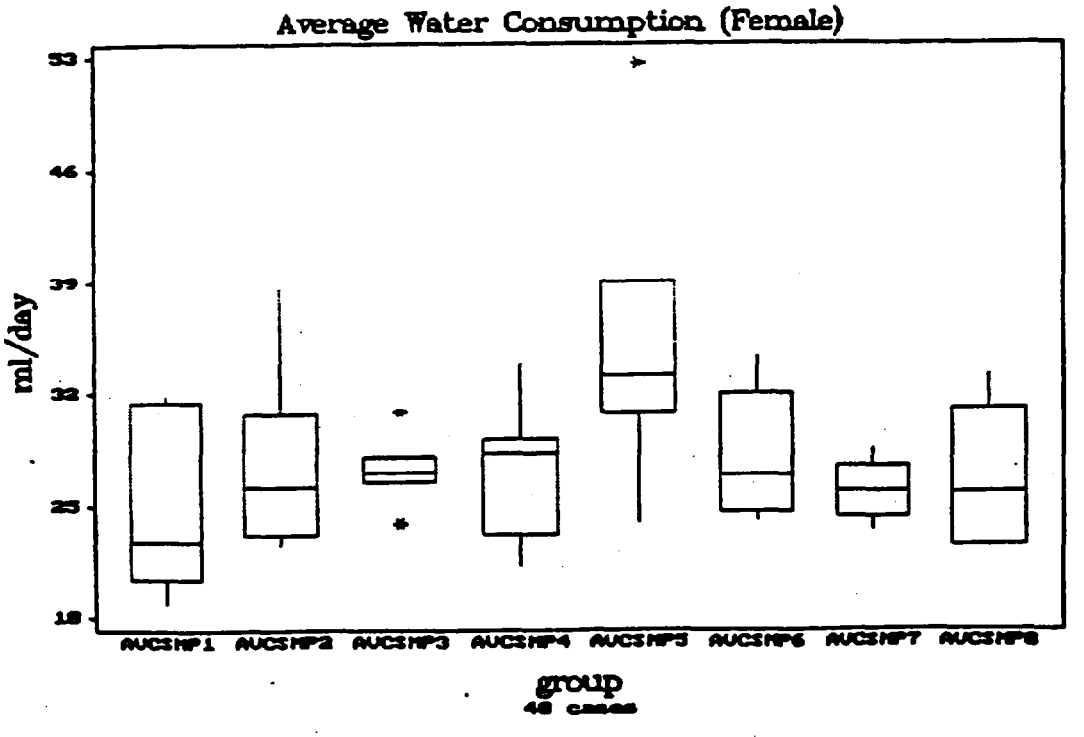

Figure 4-2. Boxplots of female rat average water consumption by dose group.

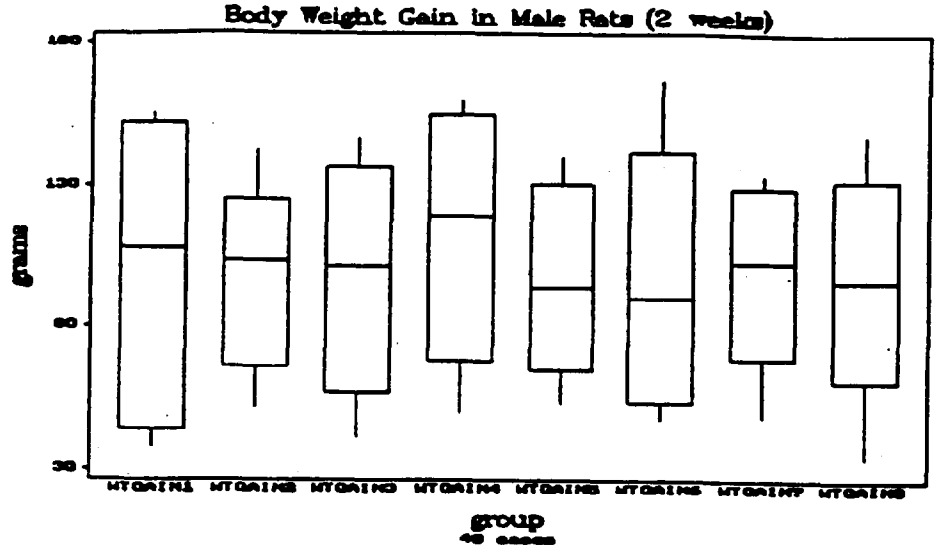

Figure 4-3. Boxplots of male rat body weight gained by dose group.

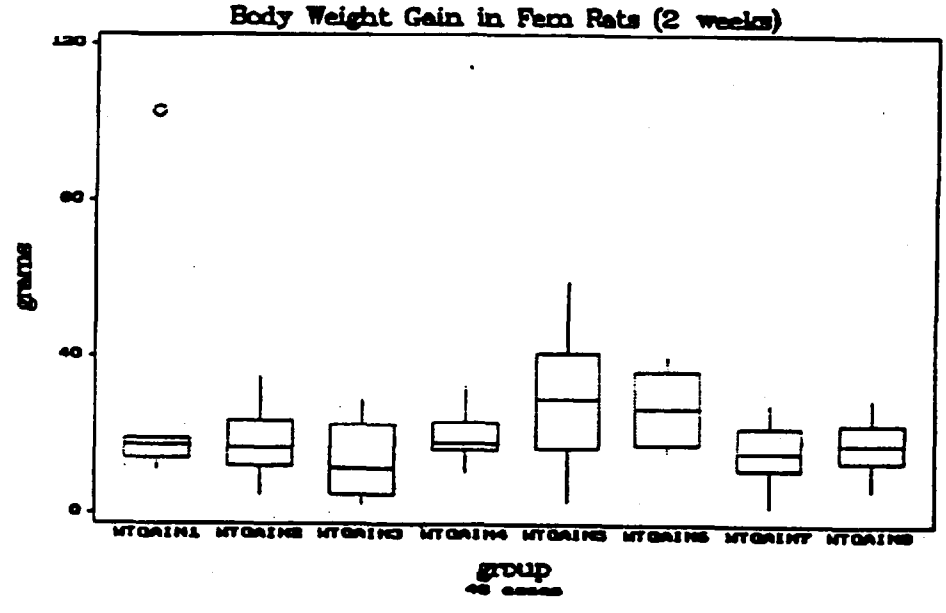

Figure 4-4. Boxplots of female rat body weight gained by dose group.

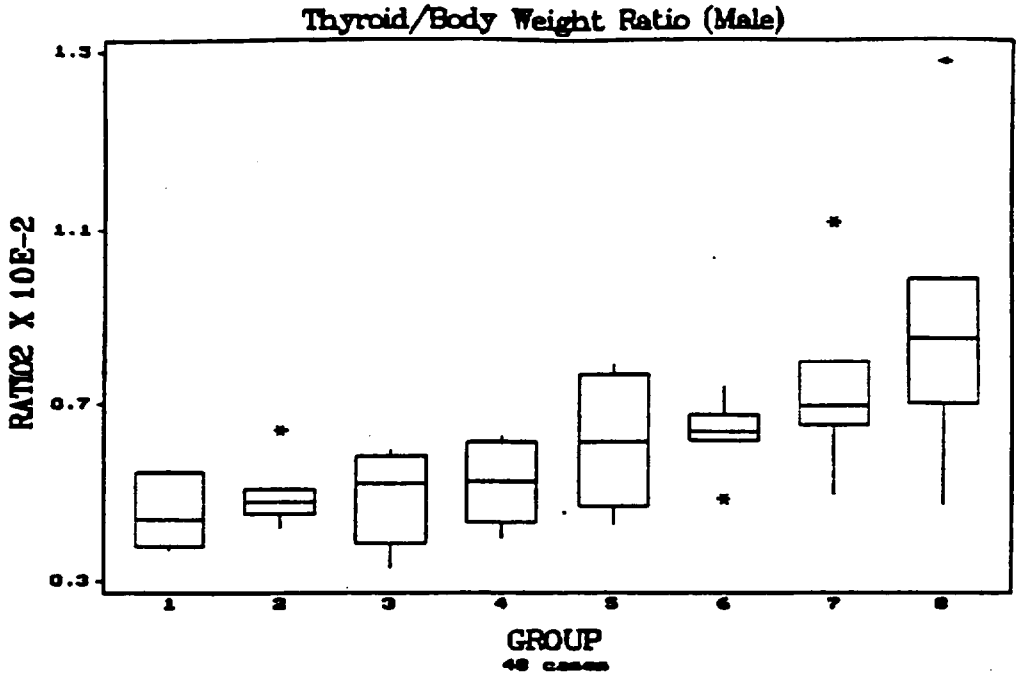

Figure 4-5. Boxplots of male thyroid/body weight ratios by dose group.

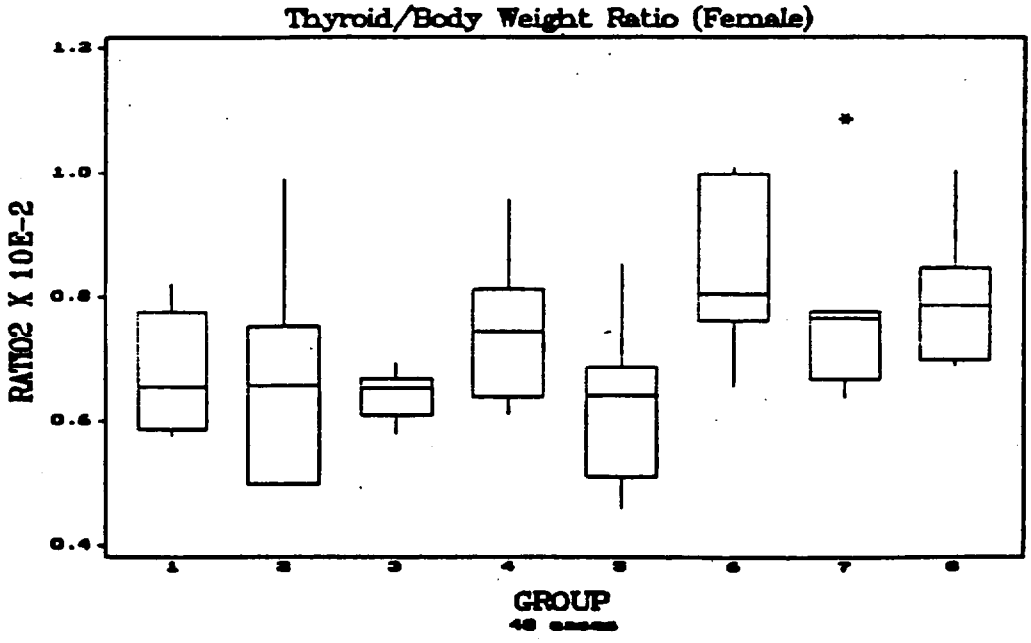

Figure 4-6. Boxplots of female thyroid/body weight ratios by dose group.

Thyroid Hormone Levels

Two-factor MANOVA was used to determine relationships between thyroid hormone levels. The results of the MANOVA are condensed in table 4-5.

Thyroid Hormone	P-Value (Dose)	P-Value (Sex)	P-Value (Dose*Sex)
Tg	.0001	.2321	.0001
rT3	.0001	.6923	.4104
T3	.0001	.0001	.0001
TSH	.0001	.0001	.0001
T4	.0006	.0001	.2909

Table 4-5. Results of two-factor MANOVA.

The results show that the null hypothesis for dose is rejected for every hormone indicating that dose does have a statistically significant impact on their levels. The sexes within dose groups were statistically significantly different in T3, TSH, and T4. In addition, the null hypotheses for interaction for Tg, T3 and TSH were rejected, indicating that the sexes were not similarly affected by the dosing. Therefore, MANOVA was used to evaluate the sexes separately.

Males

The correlation matrix for males was as follows (Fig. 4-7) (SAS output):

Correlation Analysis/Pearson Correlation Coefficients

	HTG	RT3	T3	TSH	T4
HTG	1.00000				
	0.0				
RT3	0.82190	1.00000			
	0.0001	0.0			
T3	-0.77049	-0.76134	1.00000		
	0.0001	0.0001	0.0		
TSH	0.83526	0.80132	-0.88498	1.00000	
	0.0001	0.0001	0.0001	0.0	
T4	-0.45956	-0.35538	0.21192	-0.34479	1.00000
	0.0010	0.0132	0.1482	0.0164	0.0

Figure 4-7. Correlation matrix for male rats.

Thyroglobulin (Tg)

Tg increased consistently with dose (Figs. 4-9 and 4-10). Since iodized Tg is needed to make MIT and DIT, which combine to form T3 and T4, the negative feedback mechanism could have triggered a response to produce more Tg based on declining T3 levels.

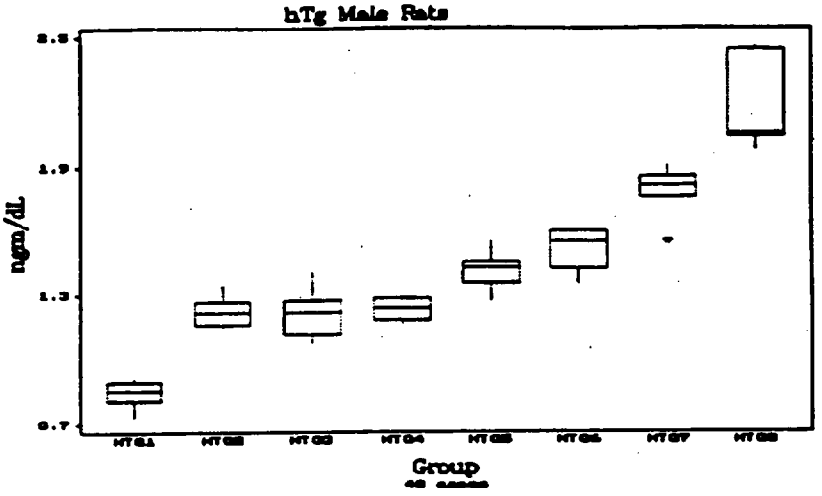

Figure 4-9. Boxplots of male Tg levels by dose group.

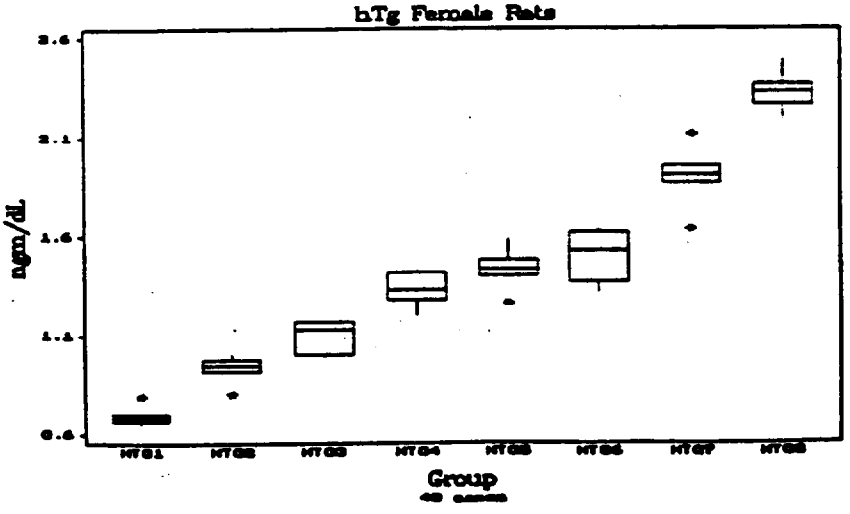

Figure 4-10. Boxplots of female Tg levels by dose group.

Thyroxine (T4)

Although T4 showed a statistically significant effect, there was no dose-response relationship (Figs. 4-13 and 4-14).

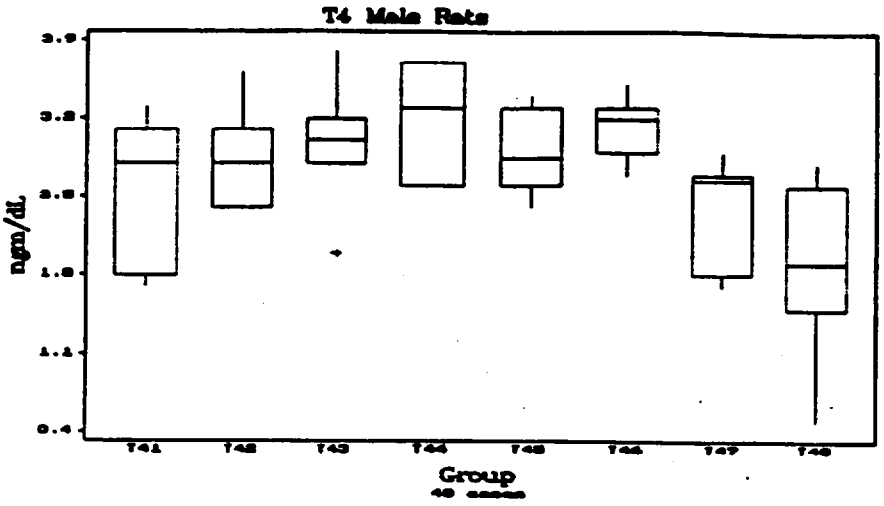

Figure 4-13. Boxplots of male T4 levels by dose group.

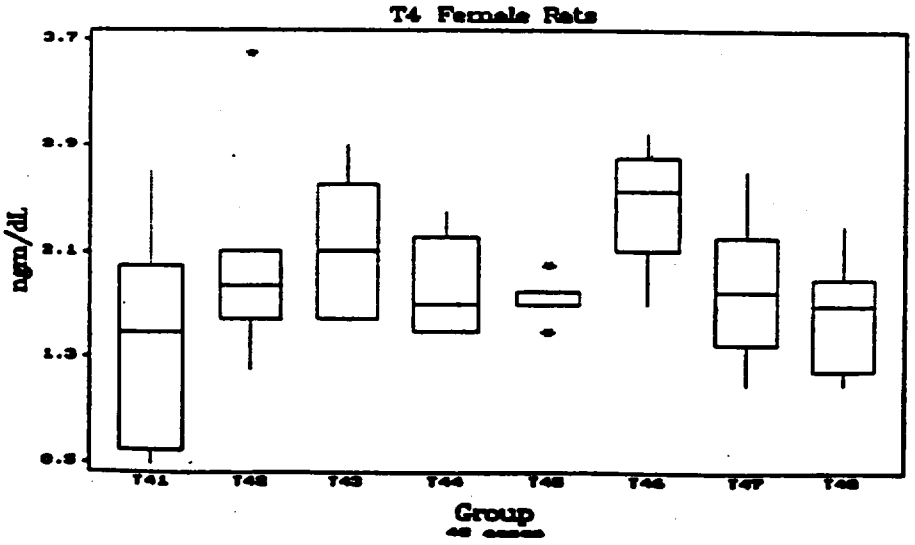

Figure 4-14. Boxplots of female T4 levels by dose group.

Females

The MLE sigmoid function for female T3 levels was as follows:

$$T3Female(Dose) = 138.364 - \frac{132.05\,Dose^{.119}}{\left[Dose^{.119} + \left(\frac{132.05}{2}\right)^{.119}\right]}$$

The relationship of the function to the data is displayed in figure 4-17.

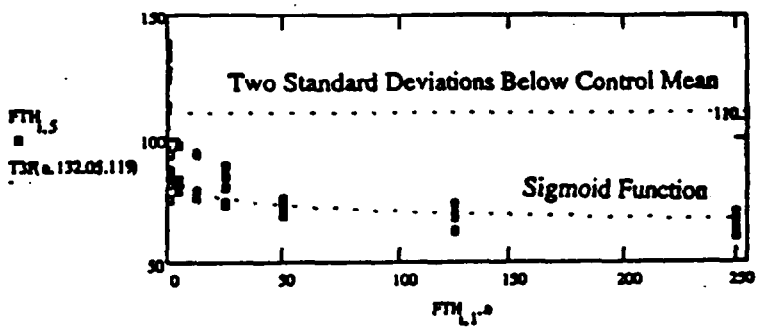

Figure 4-17. T3 dose-response data for females with fitted sigmoid function.

The lack of fit test for the female T3 sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	1369.845284	34.2461321	3.709	2.34
SS Lack of Fit	6	762.156736	127.026		
SS Error	46	2132.00202			

Table 4-6. Table for T3 sigmoid function lack of fit test (females)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 3.709 > 2.34, the null hypothesis was rejected in favor of the alternate. Therefore, the function did not accurately characterize the data and could not be used to obtain a 95% confidence interval.

Thyroid Stimulating Hormone (TSH)

A very clear relationship between dose and TSH levels was observed (Figs. 4-19 and 4-20). The mean value for TSH in the control group for females was 11.251 ngm/ml with a standard deviation of .4780. The mean value for TSH in the control group for males was 14.472 ngm/ml with a standard deviation of 1.1547.

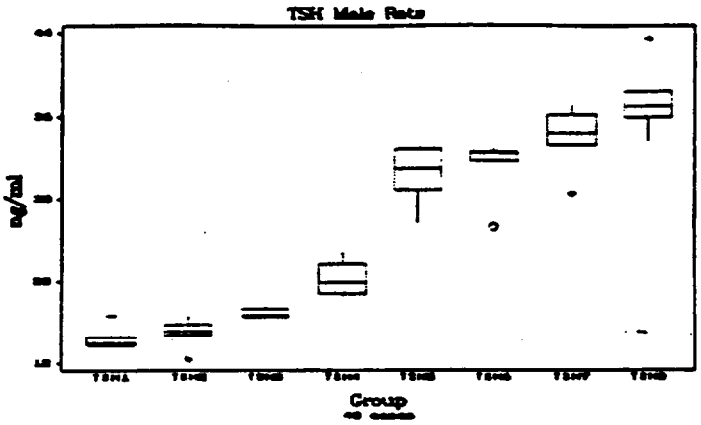

Figure 4-19. Boxplots of male TSH levels by dose group.

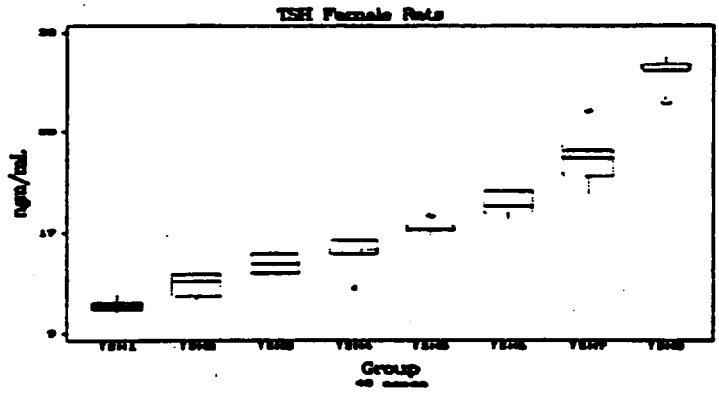

Figure 4-20. Boxplots of female TSH levels by dose group.

Males

The sigmoidal function for male TSH is as follows:

$$TSHMale(Dose) := 14.473 + \frac{20.257 \cdot Dose^{1.417}}{\left[Dose^{1.417} + \left(\frac{20.257}{2}\right)^{1.417}\right]}$$

The relationship of the function to the data is displayed in figure 4-22.

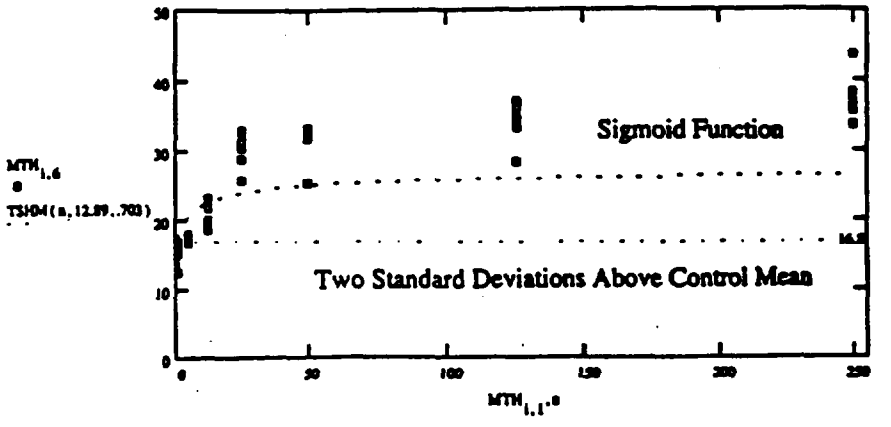

Figure 4-22. TSH dose-response data for males with fitted sigmoid function.

The lack of fit test for the male TSH sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	218.8194058	11.911	10.0262	2.34
SS Lack of Fit	6	329.090418	54.848403		
SS Error	46	547.9098236			

Table 4-9. Table for TSH sigmoid function lack of fit test (males)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 10.02 > 2.34, the null hypothesis was rejected in favor of the alternate. Therefore, the function did not accurately characterize the data.

An upper 95% confidence interval for the sigmoid function could not be determined because the functions were not able to accurately characterize the data.

Tukey's test for multiple comparisons for male T3 levels revealed that groups one and two were statistically the same (Table 4-11).

STATISTIX 4.1 HORMMALE

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF T3 BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
0	132.87	I
1	124.02	I
5	105.67	.. I *
12	90.459	... I
25	75.417	 I
50	70.690	 I
125	66.465	... I
250	65.936	... I

THERE ARE 4 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	14.674		
STANDARD ERROR FOR COMPARISON	4.5909		

Table 4-11. Tukey's test for multiple comparisons for male T3 levels.

The NOAEL in this experiment for T3 in male rats was .11 mg/kg/day.

Tukey's test for multiple comparisons for male rats revealed that dose groups one, two and three were statistically the same (Table 4-13).

STATISTIX 4.1 HORMMALE

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF TSH BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
250	37.444	I
125	33.960	II
50	31.147	-I
25	30.236	-I
12	20.250	--I*
5	16.919	--II
1	15.022	---I
0	14.472	---I

THERE ARE 4 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	4.3162		
STANDARD ERROR FOR COMPARISON	1.3504		

Table 4-13. Tukey's test for multiple comparisons for male TSH levels.

Therefore, the NOAEL in this experiment for TSH in male rats is .44 mg/kg/day.

A summary of the NOAELs is presented in table 4-14.

Dose Groups Statistically Significantly Equal to the Control		
	MALE	FEMALE
T3	25 .11 mg/kg/day	None
TSH	5 .44 mg/kg/day	125 .124 mg/kg/day

Table 4-14. Summary of NOAELs for T3 and TSH.

A summary of the results, conclusions and recommendations are presented in chapter 5.

Thyroid/Body weight ratio

AP had a statistically significant affect on the thyroid/body weight ratios of the rats exposed 11.4 mg/kg/day and higher. The thyroid/body weights in these dose groups experienced an increase in thyroid body weight ratios as compared with the control group.

Thyroid hormone levels

AP had a statistically significant affect on the thyroid hormone levels in both sexes and the sexes were not affected in the same way. Triiodothyronine (T3) levels in male and female rats fell, Thyroxine (T4) levels remained relatively unchanged. Reverse Triiodothyronine (rT3), Thyrotropin (TSH), and Thyroglobulin (Tg) all increased in both males and females.

Data

The data derived from the two-week study could not be used to establish a dose-response function in order to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group. It appeared that the dose range was not optimum and that lower doses were needed. However, a Tukey comparison of means revealed a NOAEL of .44 mg/kg/day for the male rats and .124 mg/kg/day for the female rats. These results are consistent with the assumption made by Dollarhide (1992) that .14 mg/kg/day was the NOAEL for perchlorate. Based on these NOAELs a RfD of 4.133×10^{-4} is recommended. This reference dose proposes an uncertainty factor of 300. Ten for the use of less than a chronic study, ten for the protection of sensitive individuals and three for the application of animal data to humans.

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